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## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

OFFICIAL

In re application of: Haugland *et al.*

Serial No.: 10/005,050

Filed: December 3, 2001

For: Labeling Immobilized Proteins with  
Dipyrometheneboron Difluoride Dyes

Examiner: Mary E. Ceperley

Group Art Unit: 1641

MARKED-UP VERSION OF THE CLAIMSCommissioner for Patents  
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Dear Sir:

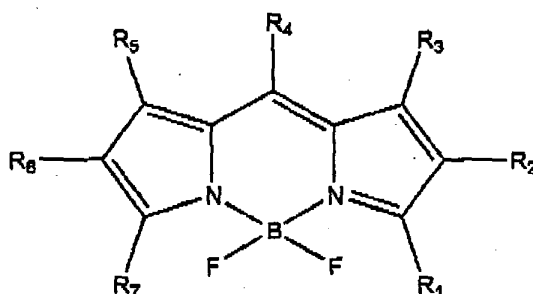
The following Marked-up Version of the Claims is hereby submitted together with the Response to Office Action on or before the due date of July 2, 2004. The Examiner is respectfully requested to enter the amendments.

## CERTIFICATE OF TRANSMISSION

I HEREBY CERTIFY THAT THIS PAPER AND THE DOCUMENTS REFERRED AS BEING ATTACHED OR ENCLOSED HERewith ARE BEING FACSIMILE TRANSMITTED TO THE UNITED STATES PATENT AND TRADEMARK OFFICE ON July 2, 2004 TO 1.703.872.9306  
By [Signature]

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1. (Currently Amended) A method of labeling poly(amino acids) comprising the steps of:
  - a. separating poly(amino acids) by gel electrophoresis, resulting in separated poly (amino acids);
  - b. transferring said separated poly(amino acids) to a solid support, resulting in immobilized poly(amino acids) ;
  - c. combining said immobilized poly(amino acids) on said solid support with a labeling mixture that comprises one or more chemically reactive dipyrrometheneboron difluoride dyes of the formula:



wherein each of R<sup>1</sup> through R<sup>7</sup> are independently selected from the group consisting of H, halogen, ~~L-Rx~~, maleimide, amine-reactive group, and substituted or unsubstituted C<sub>1</sub>-C<sub>6</sub> alkyl [~~carboxylic acid, sulfonic acid, or halogen~~], substituted or unsubstituted aryl, substituted or unsubstituted arylethenyl, substituted or unsubstituted arylbutadienyl, and substituted or unsubstituted heteroaryl [~~C<sub>1</sub>-C<sub>6</sub> alkyl, C<sub>1</sub>-C<sub>6</sub> perfluoroalkyl, cyano, halogen, azido, carboxylic acid, sulfonic acid, or halomethyl~~];  
provided that one or more of R<sup>1</sup> through R<sup>7</sup> is H,  
two or more of R<sup>1</sup> through R<sup>7</sup> is nonhydrogen, and  
wherein at least only one of R<sup>1</sup> through R<sup>7</sup> is a maleimide or an amine-reactive group -L-Rx, where L is a spacer having 1-24 nonhydrogen atoms selected from the group consisting of C, N, O, P, and S and is composed of any combination of single, double, triple or aromatic carbon-carbon bonds, carbon-nitrogen bonds, nitrogen-nitrogen

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~~bonds, carbon-oxygen bonds, carbon-sulfur bonds, phosphorus-oxygen bonds, and phosphorus-nitrogen bonds; and Rx is a reactive group that is a maleimide or a succinimidyl ester of a carboxylic acid;~~  
~~such that the dipyrrometheneboron difluoride dye has an absorption maximum between 495 nm and 640 nm;~~

d. incubating the immobilized poly(amino acids) in the labeling mixture for a sufficient time for the dyes to form a covalent bond with said poly(amino acids), resulting in labeled poly(amino acids).

2. (Currently Amended) A method, as claimed in Claim 1, wherein ~~for the dipyrrometheneboron difluoride dye, Rx is said amine-reactive group is a succinimidyl ester of a carboxylic acid.~~

3. (Original) A method, as claimed in Claim 1, wherein said solid support is made of solvent-resistant materials that are selected from the group consisting of nylon, poly(vinylidene difluoride), glass, plastics, and their derivatives.

4. (Currently Amended) A method, as claimed in Claim 3, wherein said solid support is ~~made of materials that are poly(vinylidene difluoride)~~.

5. (Cancelled) A method, as claimed in Claim 1, wherein said poly(amino acids) immobilized on said solid support has a molecular weight of 500 to-200,000 Daltons.

6. (Currently Amended) A method, as claimed in Claim [[1]] 2, wherein said dye is present in the labeling mixture at a concentration of ~~0.10 micromolar to 10 micromolar~~ about 5 micromolar to about 20 micromolar.

7. (Cancelled) A method, as claimed in Claim 1, wherein for the dipyrrometheneboron difluoride dye, R<sup>1</sup> is methyl or -L-Rx; R<sup>2</sup> is H, bromine, or -L-Rx; R<sup>3</sup> is H or methyl; R<sup>4</sup> is H or -L-Rx; R<sup>5</sup> is H, methyl, or phenyl; R<sup>6</sup> is H or bromine; and R<sup>7</sup> is methyl, phenyl, alkoxyphenyl, phenylethenyl, phenylbutadienyl pyrrolyl, or thienyl;  
where -L- is -(CH<sub>2</sub>)<sub>2</sub>-, -(CH<sub>2</sub>)<sub>4</sub>-, -OCH<sub>2</sub>C(O)NH(CH<sub>2</sub>)<sub>5</sub>-, -(CH<sub>2</sub>)<sub>2</sub>-C(O)NH(CH<sub>2</sub>)<sub>5</sub>-,

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$-(CH_2)_2C_6H_4OCH_2C(O)NH(CH_2)_5-$ ;

and Rx is a succinimidyl ester of a carboxylic acid.

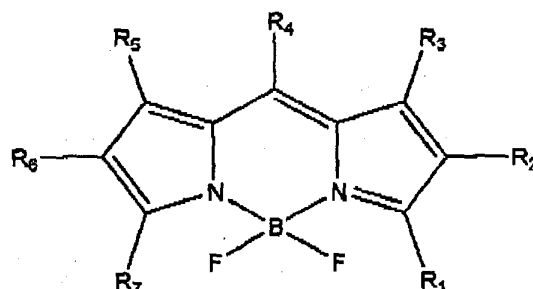
8. (Currently Amended) A method, as claimed in Claim [[7]] 1, further comprising adding a specific binding pair member that contains a label and that binds selectively to a target within the immobilized poly(amino acids) that is its complementary binding pair.

9. (Currently Amended) A method of labeling poly(amino acids) bound to aptamers comprising the steps of:

a. incubating immobilized aptamers with poly(amino acids) for a sufficient time to allow said poly(amino acids) to bind to their specific aptamers, resulting in immobilized poly(amino acids);

b. removing unbound poly(amino acids) that are not immobilized,

c. combining said immobilized poly(amino acids) with a labeling mixture that comprises one or more chemically reactive dipyrrometheneboron difluoride dyes of the formula:



wherein each of R<sup>1</sup> through R<sup>7</sup> are independently selected from the group consisting of H, halogen, L-Rx, maleimide, amine-reactive group, and substituted or unsubstituted C<sub>1</sub>-C<sub>8</sub> alkyl, substituted or unsubstituted aryl, substituted or unsubstituted arylethenyl, substituted or unsubstituted arylbutadienyl, and substituted or unsubstituted heteroaryl; provided that one or more of R<sup>1</sup> through R<sup>7</sup> is H, two or more of R<sup>1</sup> through R<sup>7</sup> is nonhydrogen, and only at least one of R<sup>1</sup> through R<sup>7</sup> is a maleimide or an amine-reactive group-L-Rx, where L is a spacer having 1-24 nonhydrogen atoms selected from the group consisting

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~~of C, N, O, P, and S and is composed of any combination of single, double, triple or aromatic carbon-carbon bonds, carbon-nitrogen bonds, nitrogen-nitrogen bonds, carbon-oxygen bonds, carbon-sulfur bonds, phosphorus-oxygen bonds, and phosphorus-nitrogen bonds; and Rx is a reactive group that is a maleimide or a succinimidyl ester of a carboxylic acid;~~  
such that the dipyrrometheneboron difluoride dye has an absorption maximum between 495 nm and 640 nm;

d. incubating the immobilized poly(amino acids) with the labeling mixture for a sufficient time to form a covalent bond between the dipyrrometheneboron difluoride dye and said immobilized poly(amino acids), resulting in labeled poly(amino acids) that are bound to the aptamers.

10. (Currently Amended) A method, as claimed in Claim 9, wherein ~~the dipyrrometheneboron difluoride dye's chemically reactive group is said amine-reactive group~~ is a succinimidyl ester of a carboxylic acid.

11. (Currently Amended) A method, as claimed in Claim [[9]] 10, wherein said dipyrrometheneboron difluoride dye is present in the ~~combined~~ labeling mixture at a concentration of ~~0-10 micromolar to 10 micromolar~~ about 5 micromolar to about 20 micromolar.

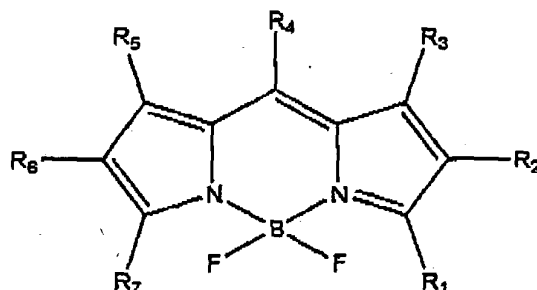
12. (Cancelled) A method, as claimed in Claim 9, wherein for the dipyrrometheneboron difluoride dye, R<sup>1</sup> is methyl or -L-Rx; R<sup>2</sup> is H, bromine, or -L-Rx; R<sup>3</sup> is H or methyl; R<sup>4</sup> is H or -L-Rx; R<sup>5</sup> is H, methyl, or phenyl; R<sup>6</sup> is H or bromine; and R<sup>7</sup> is methyl, phenyl, alkoxyphenyl, phenylethenyl, phenylbutadienyl, pyrrolyl, or thienyl;  
where -L- is -(CH<sub>2</sub>)<sub>2</sub>-, -(CH<sub>2</sub>)<sub>4</sub>-, -OCH<sub>2</sub>C(O)NH(CH<sub>2</sub>)<sub>5</sub>-, -(CH<sub>2</sub>)<sub>2</sub>-C(O)NH(CH<sub>2</sub>)<sub>5</sub>-,  
(CH)<sub>2</sub>C<sub>6</sub>H<sub>4</sub>OCH<sub>2</sub>C(O)NH(CH<sub>2</sub>)<sub>5</sub>-;  
and Rx is a succinimidyl ester of a carboxylic acid.

13. (Original) A method, as claimed in Claim 12, further comprising adding a specific binding pair member that contains a label and that binds selectively to a target within the immobilized poly(amino acids) that is its complementary binding pair.

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14. (Currently Amended) A method of labeling immobilized poly(amino acids) in an array comprising the steps of:

a. combining an array of immobilized poly(amino acids) with a labeling mixture that comprises one or more chemically reactive dipyrrometheneboron difluoride dyes of the formula



wherein each of R<sup>1</sup> through R<sup>7</sup> are independently selected from the group consisting of H, halogen, L-Rx, maleimide, amine-reactive group, and substituted or unsubstituted C<sub>1</sub>-C<sub>6</sub> alkyl, substituted or unsubstituted aryl, substituted or unsubstituted arylethenyl, substituted or unsubstituted arylbutadienyl, and substituted or unsubstituted heteroaryl; provided that one or more of R<sup>1</sup> through R<sup>7</sup> is H, two or more of R<sup>1</sup> through R<sup>7</sup> is nonhydrogen, and only at least one of R<sup>1</sup> through R<sup>7</sup> is a maleimide or an amine-reactive group -L-Rx, where L is a spacer having 1-24 nonhydrogen atoms selected from the group consisting of C, N, O, P, and S and is composed of any combination of single, double, triple or aromatic carbon-carbon bonds, carbon-nitrogen bonds, nitrogen-nitrogen bonds, carbon-oxygen bonds, carbon-sulfur bonds, phosphorus-oxygen bonds, and phosphorus-nitrogen bonds; and Rx is a reactive group that is a maleimide or a succinimidyl ester of a carboxylic acid; such that the dipyrrometheneboron difluoride dye has an absorption maximum between 495 nm and 640 nm;

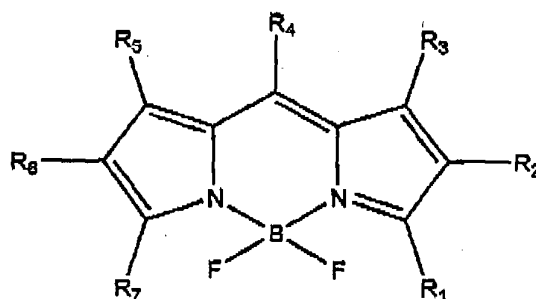
b. incubating said array with the labeling mixture for a sufficient time to form a covalent bond between the dipyrrometheneboron difluoride dye and said immobilized poly(amino acids),

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resulting in the array of poly(amino acids) being labeled.

15. (Currently Amended) A method, as claimed in Claim 14, wherein for the ~~dipyrrrometheneboron difluoride dye, Rx is~~ said amine-reactive group is a succinimidyl ester of a carboxylic acid.
16. (Currently Amended) A method, as claimed in Claim ~~[[14]]~~ 15, wherein said dipyrrrometheneboron difluoride dye is present in the labeling mixture at a concentration of 0-10 ~~micromolar~~ 10 micromolar ~~about 5 micromolar to about 20 micromolar.~~
17. (Cancelled) A method, as claimed in Claim 14, wherein for the dipyrrrometheneboron difluoride dyes, R<sup>1</sup> is methyl or -L-Rx; R<sup>2</sup> is H, bromine, or -L-Rx; R<sup>3</sup> is H or methyl; R<sup>4</sup> is H or -L-Rx; R<sup>5</sup> is H, methyl, or phenyl; R<sup>6</sup> is H or bromine; and R<sup>7</sup> is methyl, phenyl, alkoxyphenyl, phenylethenyl, phenylbutadienyl pyrrolyl, or thienyl;  
where -L- is -(CH<sub>2</sub>)<sub>2</sub>-, -(CH<sub>2</sub>)<sub>4</sub>-, -OCH<sub>2</sub>C(O)NH(CH<sub>2</sub>)<sub>5</sub>-, -(CH<sub>2</sub>)<sub>2</sub>-C(O)NH(CH<sub>2</sub>)<sub>5</sub>-,  
(CH)<sub>2</sub>C<sub>6</sub>H<sub>4</sub>OCH<sub>2</sub>C(O)NH(CH<sub>2</sub>)<sub>5</sub>-;  
and Rx is a succinimidyl ester of a carboxylic acid.
18. (Cancelled) A method, as claimed in Claim 17, further comprising adding specific binding pair member that contains a label and that binds selectively to a target within the immobilized poly(amino acids) that is its complementary binding pair.
19. (Original) A method, as claimed in Claim 14, further comprising adding specific binding pair member that contains a label and that binds selectively to a target within the immobilized poly(amino acids) that is its complementary binding pair.
20. (Currently Amended) A method of detecting poly(amino acids) comprising the steps of:
- a. combining poly(amino acids) immobilized on a solid support; with a labeling mixture that comprises one or more chemically reactive dipyrrrometheneboron difluoride dyes of the formula

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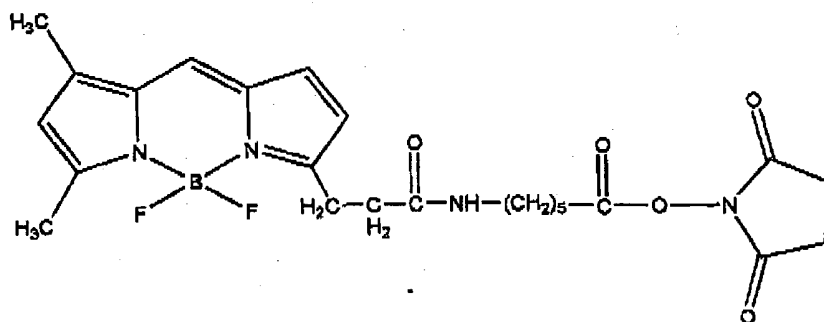


wherein each of  $R^1$  through  $R^7$  are independently selected from the group consisting of H, halogen,  $-R_x$ , maleimide, amine-reactive group, and substituted or unsubstituted  $C_1$ - $C_6$  alkyl, substituted or unsubstituted aryl, substituted or unsubstituted arylethenyl, substituted or unsubstituted arylbutadienyl, and substituted or unsubstituted heteroaryl; provided that one or more of  $R^1$  through  $R^7$  is H, two or more of  $R^1$  through  $R^7$  is nonhydrogen, and only at least one of  $R^1$  through  $R^7$  is a maleimide or an amine-reactive group  $-R_x$ , where L is a spacer having 1-24 nonhydrogen atoms selected from the group consisting of C, N, O, P, and S and is composed of any combination of single, double, triple or aromatic carbon-carbon bonds, carbon-nitrogen bonds, nitrogen-nitrogen bonds, carbon-oxygen bonds, carbon-sulfur bonds, phosphorus-oxygen bonds, and phosphorus-nitrogen bonds; and  $R_x$  is a reactive group that is a maleimide or a succinimidyl ester of a carboxylic acid; such that the dipyrrometheneboron difluoride dye has an absorption maximum between 495 nm and 640 nm

- b. incubating said immobilized poly(amino acids) with the labeling mixture for a sufficient time to form a covalent bond between the dipyrrometheneboron difluoride dye and said immobilized poly(amino acids) resulting in labeled poly(amino acids);
- c. removing unbound dipyrrometheneboron difluoride dyes ;
- d. illuminating said labeled poly(amino acids) to yield a fluorescent optical response to detect the corresponding labeled poly(amino acids).

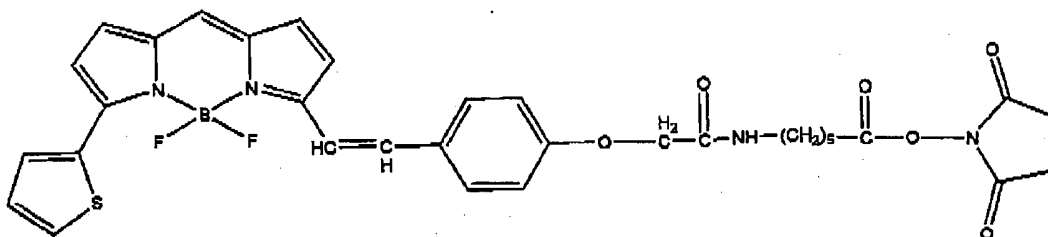


21. (Original) A method, as claimed in Claim 20, wherein the dipyrrometheneboron difluoride dye has the formula:

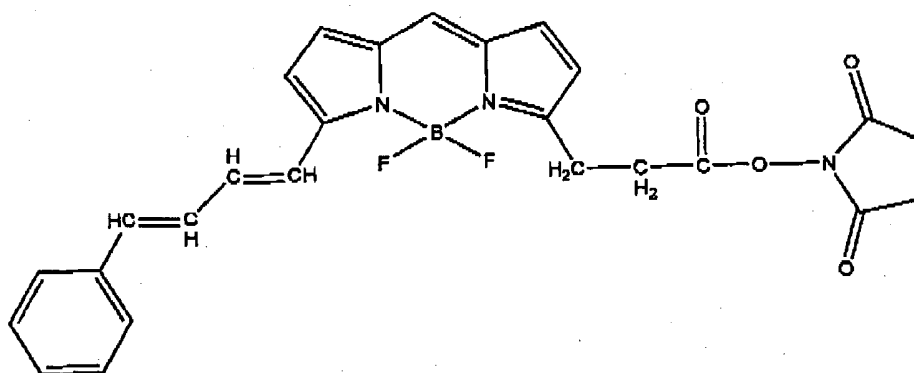
c1ccc(cc1)C2=CC=C3C(=C2)N(B(F)(F)C4=CC=CC=C4)C5=CC=CC=C35C(=C4)CC(=O)NCC(=O)OCC(=O)N6C(=O)CC(=O)N6COc1ccc(cc1)c2c3c(c4c5c(c2)c(c6c5c(c3)c(c4)C)N(B(F)(F)F)c6)CC(C)C(=O)NCC(=O)OCC7C(=O)NCC7=O

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24. (Original) A method, as claimed in Claim 20, wherein the dipyrrometheneboron difluoride dye has the formula:



25. (Original) A method, as claimed in Claim 20, wherein the dipyrrometheneboron difluoride dye has the formula:



26. (Original) A method, as claimed in Claim 20, wherein the dipyrrometheneboron difluoride dye has the formula:

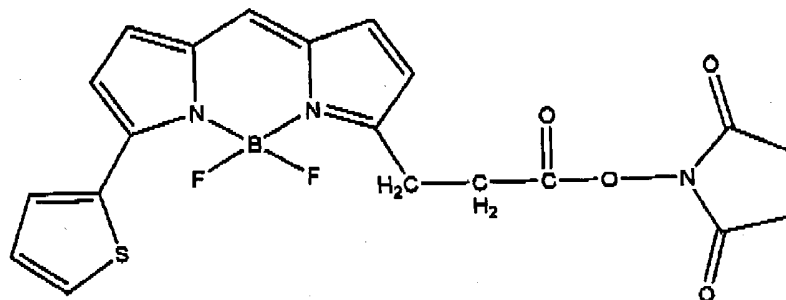
c1ccc(cc1)/C=C/c2c3ccccc3n(c2)B(F)(F)n4c5ccccc5n4CCCC(=O)O[nH]c6c(=O)c(=O)c6

Chemical structure of compound 1: A bis-indole boron complex. A central boron atom is coordinated by two fluorine atoms and two nitrogen atoms of a bis-indole system. One indole ring is substituted with a phenyl group, and the other is substituted with a 2-(2-oxo-2-oxazolidinone)ethyl group.

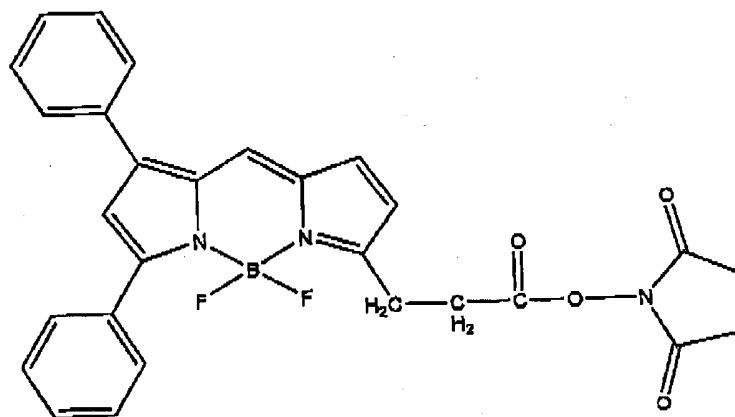
c1ccc[nH]1C2=CC=C3C(=C2)N(B(F)F)C4=CC=CC=C4N3C5=CC=CC=C5CC(=O)OCC6=CC(=O)N(C6=O)C7=CC=CC=C7

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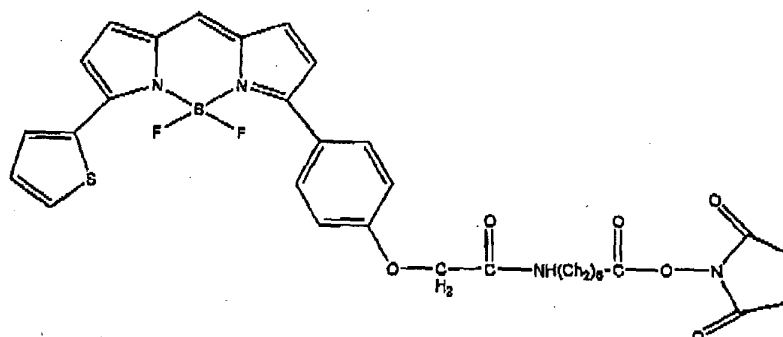


30. (Original) A method, as claimed in Claim 20, wherein the dipyrrometheneboron difluoride dye has the formula:



31. (Original) A method, as claimed in Claim 20, wherein the dipyrrometheneboron difluoride dye has the formula:

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32. (Original) A method, as claimed in Claim 20, wherein said solid support is made of solvent-resistant materials that are selected from the group consisting of nylon, poly(vinylidene difluoride), glass, plastics, and their derivatives.

33. (Currently Amended) A method, as claimed in Claim 32, wherein said solid support is made of materials that are poly(vinylidene difluoride).

34. (Cancelled) A method, as claimed in Claim 20, wherein said poly(amino acids) on said solid support each have a molecular weight of between 500 Daltons and 200,000 Daltons.

35. (Cancelled) A method, as claimed in Claim 20, wherein for said dipyrrometheneboron difluoride dye  $R^1$  is methyl or  $-L-Rx$ ;  $R^2$  is H, bromine, or  $-L-Rx$ ;  $R^3$  is H or methyl;  $R^4$  is H or  $-L-Rx$ ;  $R^5$  is H, methyl, or phenyl;  $R^6$  is H or bromine; and  $R^7$  is methyl, phenyl, alkoxyphenyl, phenylethenyl, phenylbutadienyl, pyrrolyl, or thienyl;

where  $-L-$  is  $-(CH_2)_2-$ ,  $-(CH_2)_4-$ ,  $-OCH_2C(O)NH(CH_2)_5-$ ,  $-(CH_2)_2-C(O)NH(CH_2)_5-$ ,

$(CH)_2C_6H_4OCH_2C(O)NH(CH_2)_5-$ ;

and  $Rx$  is a succinimidyl ester of a carboxylic acid..

36. (Currently Amended) A method, as claimed in Claim 35, wherein said dipyrrometheneboron difluoride dye is present in the labeling mixture at a concentration of about 5 micromolar to about 20 micromolar ~~0.10 micromolar to 10 micromolar~~, and

~~wherein said labeled poly(amino acids) are illuminated for five seconds or less.~~

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37. (Original) A method, as claimed in Claim 20, further comprising adding a specific binding pair member that selectively binds to a target within said immobilized poly(amino acids) that is its complementary binding pair.

38. (Original) A method, as claimed in Claim 37, where said specific binding pair member contains a label that is an enzyme or a hapten.

39. (Original) A method, as claimed in Claim 37, where said specific binding pair member contains a label that is a fluorophore.

40. (Original) A method, as claimed in Claim 37, further comprising:  
adding a secondary complementary binding pair member that contains a label and that selectively binds to the specific binding pair member.

41. (Original) A method, as claimed in Claim 40, wherein the label on the secondary complementary binding pair is an enzyme.

42. (Original) A method, as claimed in Claim 40, wherein the label on the secondary complementary binding pair is a fluorescent dye.

43. (Original) A method, as claimed in Claim 41, wherein said enzyme is a peroxidase or a phosphatase.

44. (Original) A method, as claimed in Claim 43, wherein said peroxidase is horseradish peroxidase.

45. (Original) A method, as claimed in Claim 43 wherein said phosphatase is alkaline phosphatase.

46. (Currently Amended) A method, as claimed in Claim 41, wherein said enzyme is capable of utilizing a fluorogenic substrate to generate a detectable optical response.

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47. (Original) A method, as claimed in Claim 46, wherein said enzyme is a peroxidase and said fluorogenic substrate is a fluorescent tyramide.

48. (Original) A method, as claimed in Claim 46, wherein said enzyme is a phosphatase and said fluorogenic substrate is a quinazolinone phosphate.

49. (Original) A method, as claimed in Claim 46, wherein said enzyme is a phosphatase and said fluorogenic substrate is 9H-(1,3-dichloro-9,9-dimethylacridin-2-one-7-yl) phosphate.

50. (Original) A method, as claimed in Claim 46, wherein said enzyme is a peroxidase and said fluorogenic substrate is a polyfluorinated xanthene.

51. (Original) A method, as claimed in Claim 40, wherein said secondary complimentary binding pair is an antibody or an antibody fragment.

52. (Original) A method, as claimed in Claim 39, wherein said complementary specific binding pair member is a lectin.

53. (Original) A method, as claimed in Claim 39, wherein said specific binding pair member is biotin-binding protein that contains a label.

54. (Original) A method, as claimed in Claim 53, wherein said biotin-binding protein is streptavidin.

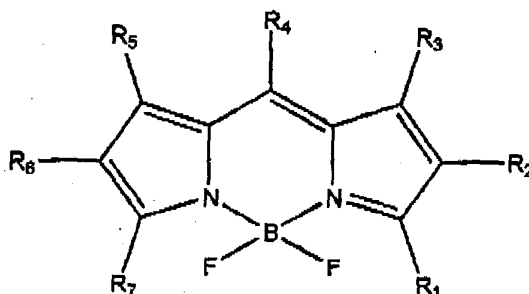
55. (Original) A method, as claimed in Claim 53, wherein said biotin-binding protein is NeutrAvidin.

56. (Original) A method, as claimed in Claim 37, wherein said specific binding pair member is an antibody or antibody fragment, an aptamer, a lectin, or a biotin-binding protein.

57. (Withdrawn) A kit for detection of poly(amino acids) immobilized on a solid surface, said kit comprising:

a. a dipyrrometheneboron difluoride dye of the formula:

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wherein each of R<sup>1</sup> through R<sup>7</sup> are independently selected from the group consisting of H, halogen, L-Rx, and substituted or unsubstituted C<sub>1</sub>-C<sub>6</sub> alkyl, aryl, arylolethynyl, arylbutadienyl, and heteroaryl;  
provided that one or more of R<sup>1</sup> through R<sup>7</sup> is H,  
two or more of R<sup>1</sup> through R<sup>7</sup> is nonhydrogen, and  
only one of R<sup>1</sup> through R<sup>7</sup> is -L-Rx, where L is a spacer having 1-24 nonhydrogen atoms selected from the group consisting of C, N, O, P, and S and is composed of any combination of single, double, triple or aromatic carbon-carbon bonds, carbon-nitrogen bonds, nitrogen-nitrogen bonds, carbon-oxygen bonds, carbon-sulfur bonds, phosphorus-oxygen bonds, and phosphorus-nitrogen bonds; and Rx is a reactive group that is a maleimide or a succinimidyl ester of a carboxylic acid;  
such that the dipyrrometheneboron difluoride dye has an absorption maximum between 495 nm and 640 nm;

b. a specific binding pair member that contains a label and that selectively binds to a target that is its complementary binding pair.

58. (Withdrawn) A kit, as claimed in Claim 57, wherein the specific binding pair member contains a label that is an enzyme; wherein said enzyme is capable utilizing a fluorogenic substrate to generate a detectable optical response, said kit further comprising the fluorogenic substrate.

59. (Withdrawn) A kit, as claimed in Claim 57, wherein said specific binding pair member is an antibody or antibody fragment.



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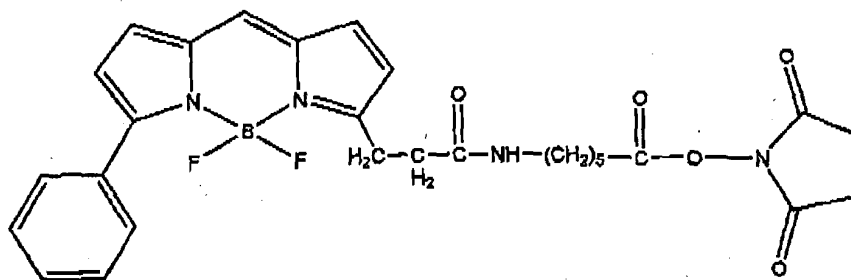
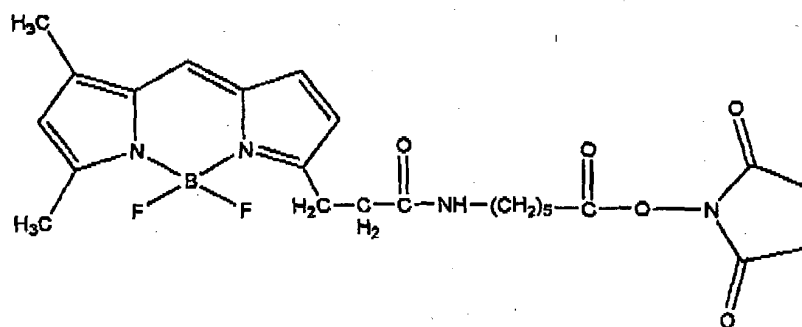
60. (Withdrawn) A kit, as claimed in Claim 57, wherein the specific binding pair member contains a label that is a fluorescent dye.
61. (Withdrawn) A kit, as claimed in Claim 57, wherein said specific binding pair member is a biotin-binding protein.
62. (Withdrawn) A kit, as claimed in Claim 61, wherein said biotin-binding protein is avidin, Neutravidin or streptavidin.
63. (Withdrawn) A kit, as claimed in Claim 58, wherein said label is an enzyme that is a peroxidase or a phosphatase.
64. (Withdrawn) A kit, as claimed in Claim 63, wherein said peroxidase is horseradish peroxidase.
65. (Withdrawn) A kit, as claimed in Claim 64, wherein said fluorogenic substrate peroxidase substrate that is a fluorescent tyramide.
66. (Withdrawn) A kit, as claimed in Claim 63, wherein said phosphatase is alkaline phosphatase.
67. (Withdrawn) A kit, as claimed in Claim 66, wherein said fluorogenic substrate is a phosphatase substrate that is a 9H-(1,3-dichloro-9,9-dimethylacridin-2-one-7-yl) phosphate.
68. (Withdrawn) A kit, as claimed in Claim 66, wherein said fluorogenic substrate is a phosphatase substrate that is a 2-(5'-chloro-2'-phosphoryloxyphenyl)-6-chloro-4(3H)-quinazolinone.
69. (Withdrawn) A kit, as claimed in Claim 66, wherein said fluorogenic substrate is a phosphatase substrate that is ELF 39 reagent.
70. (Withdrawn) A kit, as claimed in Claim 58, wherein

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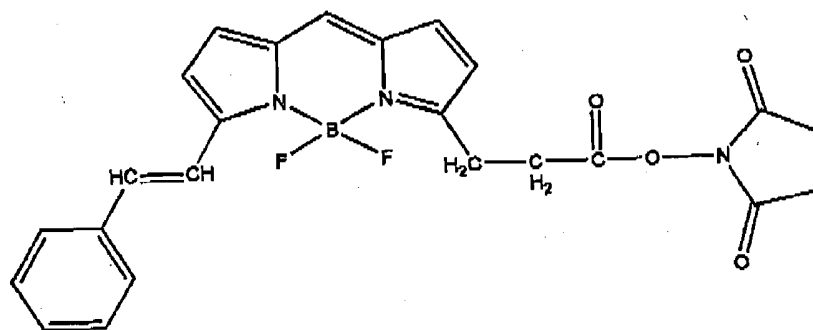
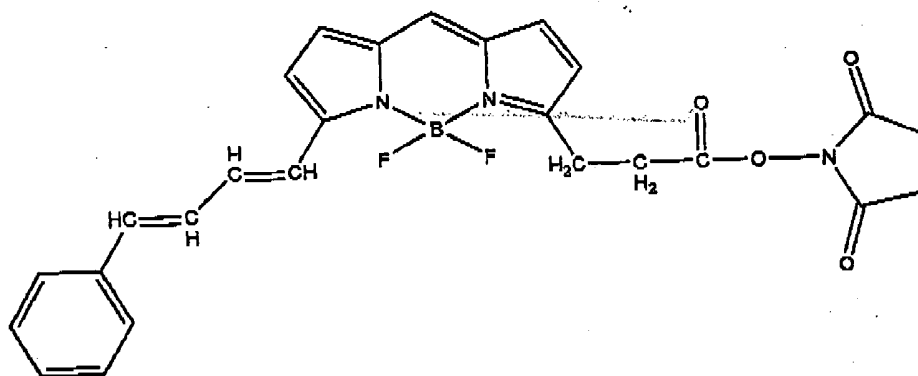
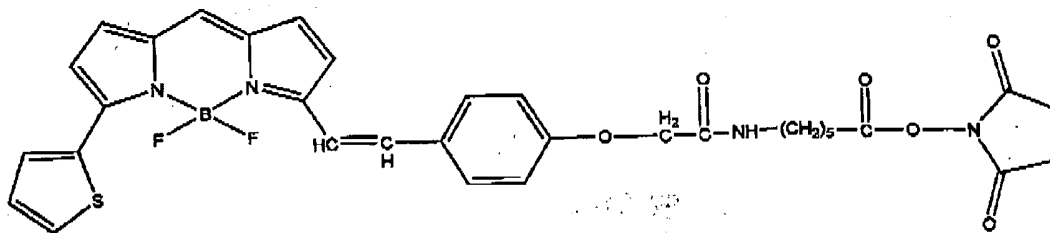
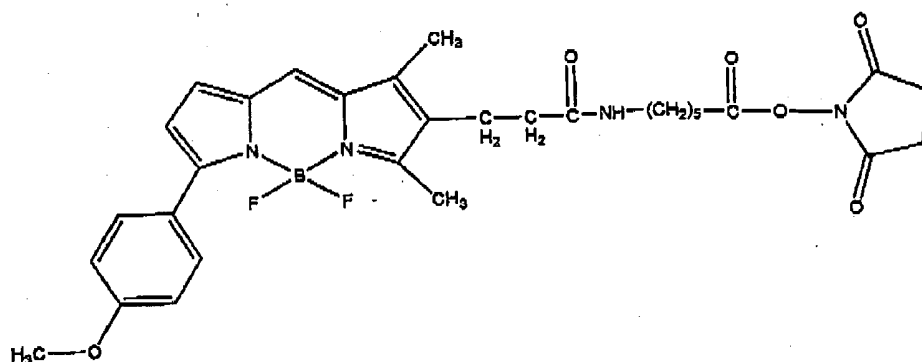
for the dipyrrometheneboron difluoride dye,  $R^1$  is methyl or  $-L-Rx$ ;  $R^2$  is H, bromine, or  $-L-Rx$ ;  $R^3$  is H or methyl;  $R^4$  is H or  $-L-Rx$ ;  $R^5$  is H, methyl, or phenyl;  $R^6$  is H or bromine; and  $R^7$  is methyl, phenyl, alkoxyphenyl, phenylethenyl, phenylbutadienyl, pyrrolyl, or thienyl;  
where  $-L-$  is  $-(CH_2)_2-$ ,  $-(CH_2)_4-$ ,  $-OCH_2C(O)NH(CH_2)_5-$ ,  $-(CH_2)_2-C(O)NH(CH_2)_5-$ ,  
 $-(CH)_2C_6H_4OCH_2C(O)NH(CH_2)_5-$ ;  
and  $Rx$  is a succinimidyl ester of a carboxylic acid;

the specific binding pair member is an antibody or a streptavidin that contains a label that is an alkaline phosphatase and the fluorogenic substrate is a 9H-(1,3-dichloro-9,9-dimethylacridin-2-one-7-yl) phosphate, a 2-(5'-chloro-2'-phosphoryloxyphenyl)-6-chloro-4(3H)-quinazolinone, or ELF 39 reagent.

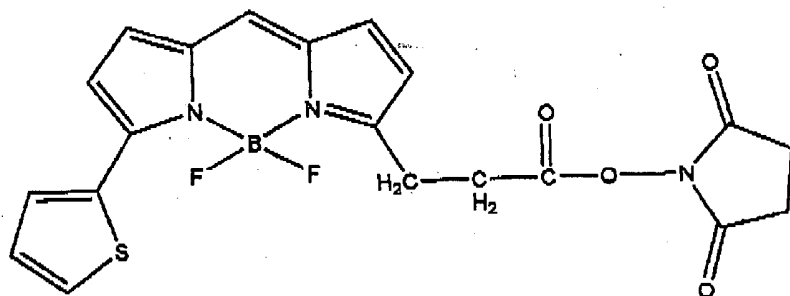
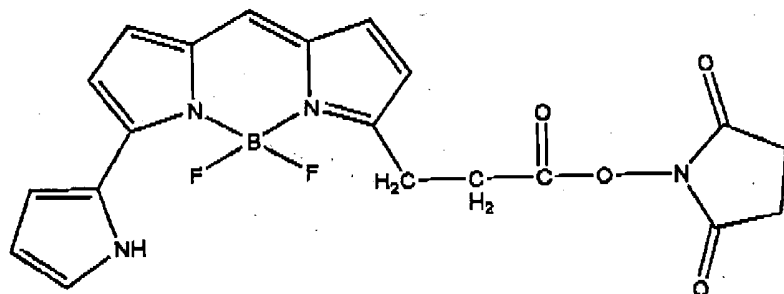
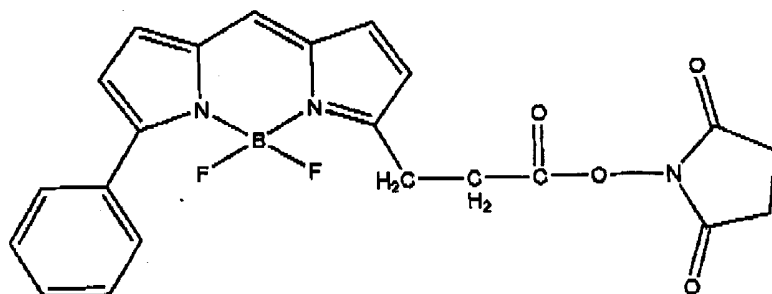
71. (New) A method, as claimed in Claim 6, wherein said dye is selected from the group consisting of



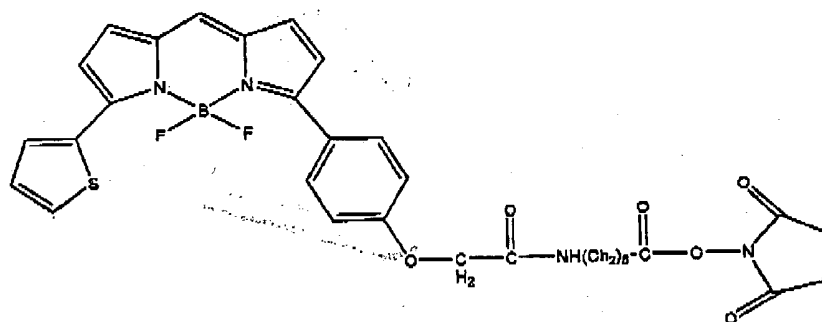
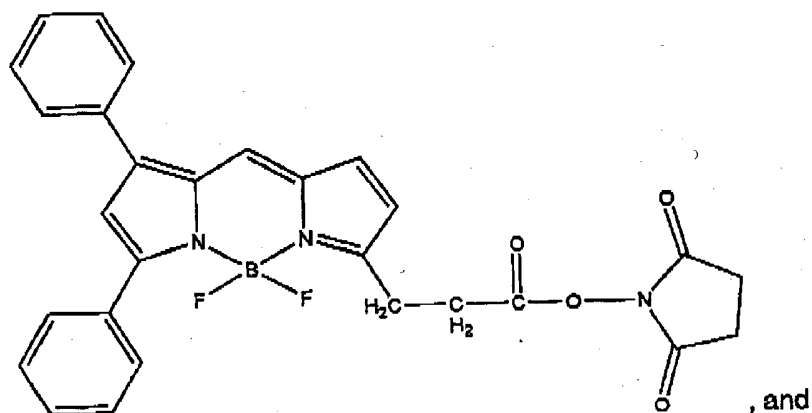
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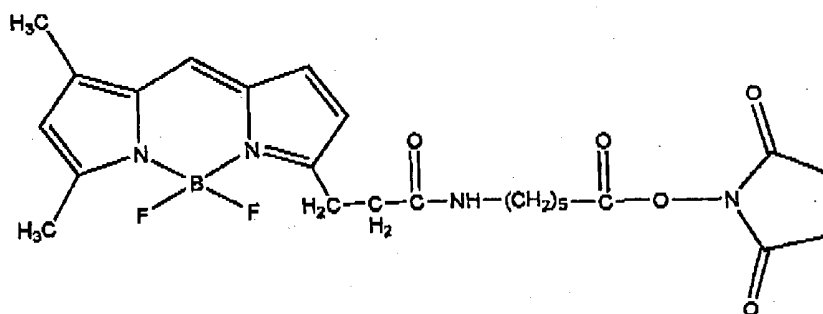
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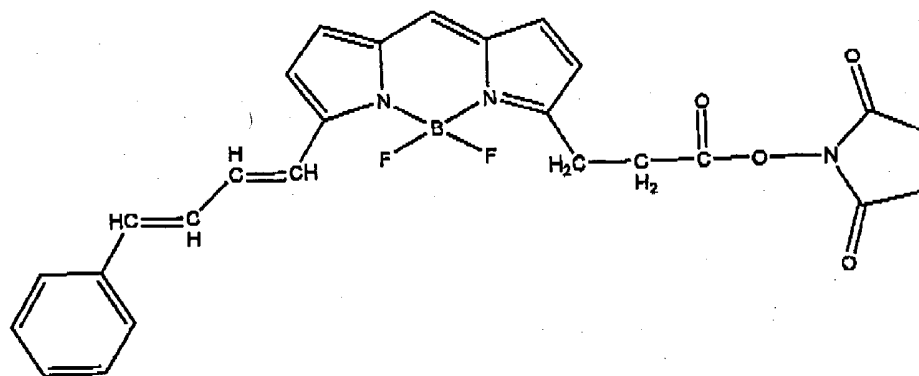
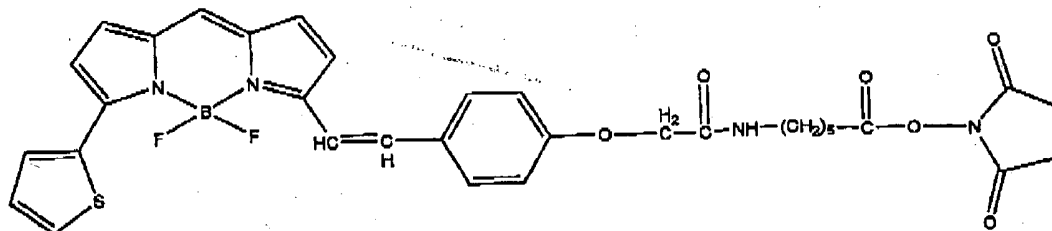
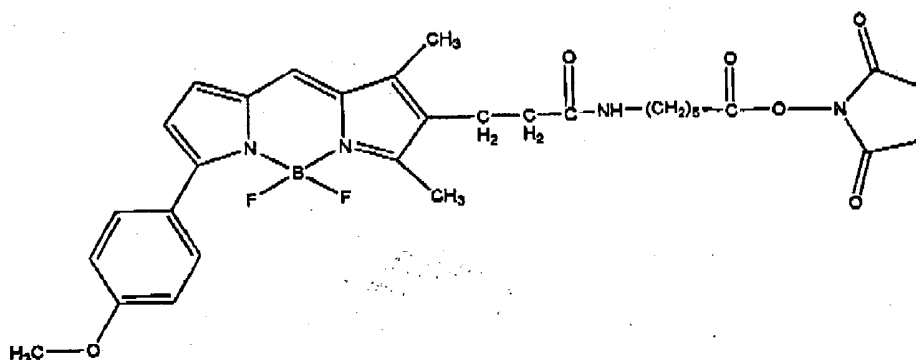
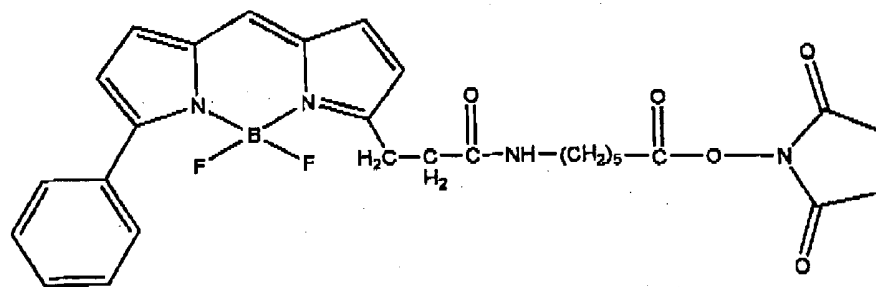
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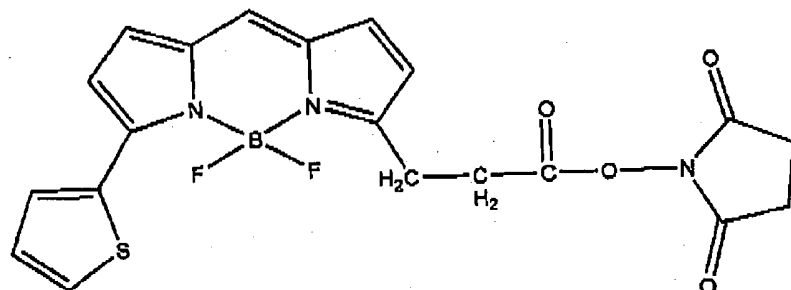
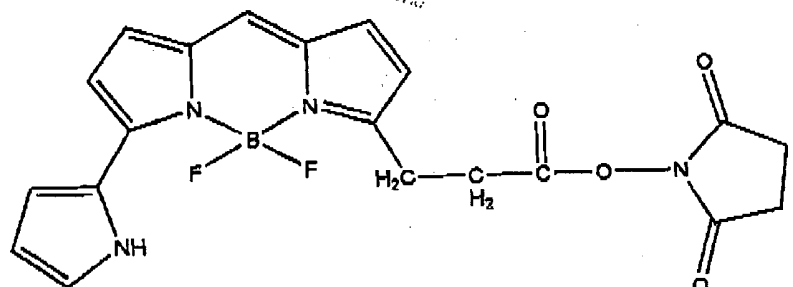
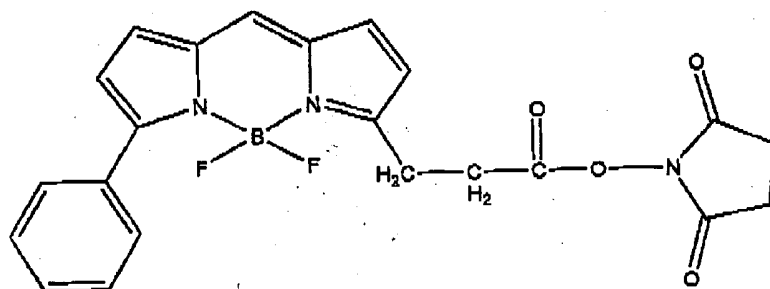
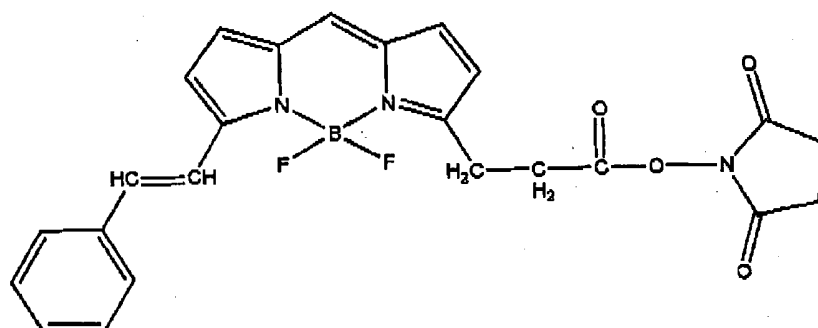
72. (New) A method, as claimed in Claim 11, wherein said dye is selected from the group consisting of



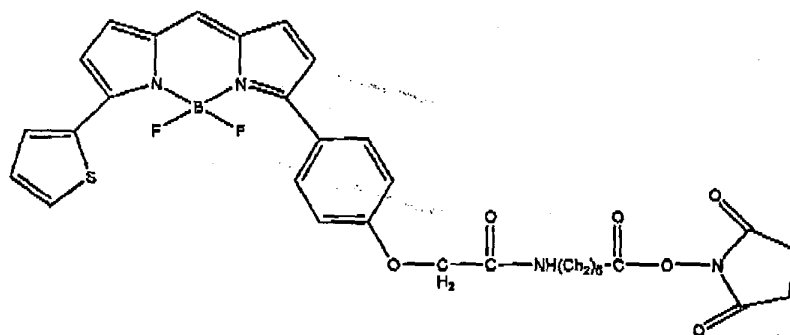
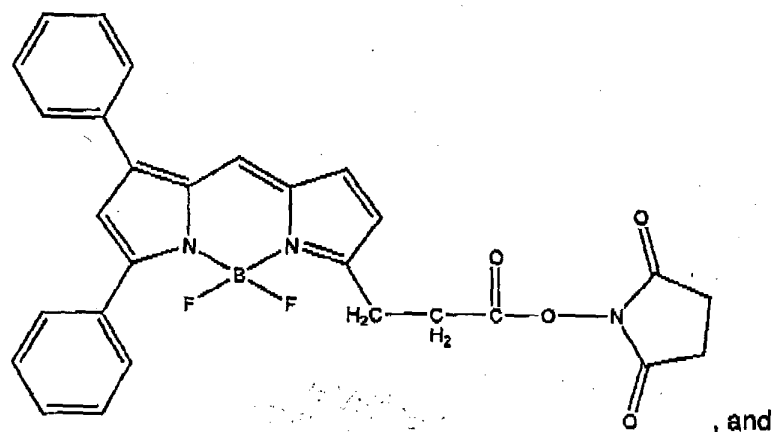
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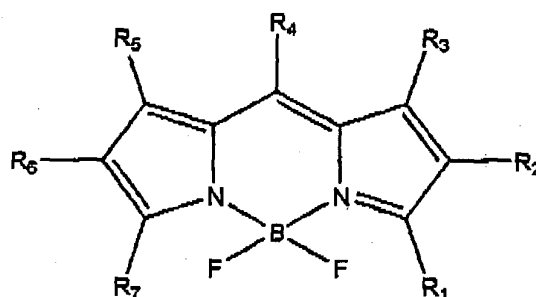
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73. (New) A method of detecting immobilized poly(amino acids) comprising the steps of:
- separating poly(amino acids) by gel electrophoresis, resulting in separated poly (amino acids);
  - transferring said separated poly(amino acids) to a solid support, resulting in immobilized poly(amino acids) ;
  - combining said immobilized poly(amino acids) on said solid support with a labeling mixture that comprises one or more chemically reactive dipyrrometheneboron difluoride dyes of the formula:



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wherein each of  $R^1$  through  $R^7$  are independently selected from the group consisting of H, halogen, maleimide, amine-reactive group, substituted or unsubstituted  $C_1$ - $C_6$  alkyl substituted or unsubstituted aryl, substituted or unsubstituted arylethenyl, substituted or unsubstituted arylbutadienyl, and substituted or unsubstituted heteroaryl;

wherein at least one of  $R^1$  through  $R^7$  is a maleimide or an amine-reactive group;

- d) incubating the immobilized poly(amino acids) in the labeling mixture for a sufficient time for the dyes to form a covalent bond with said poly(amino acids), resulting in labeled poly(amino acids);
- e) removing unbound dipyrrometheneboron difluoride dyes;
- f) illuminating said labeled poly(amino acids) to yield a fluorescent optical response to detect the corresponding labeled poly(amino acids).

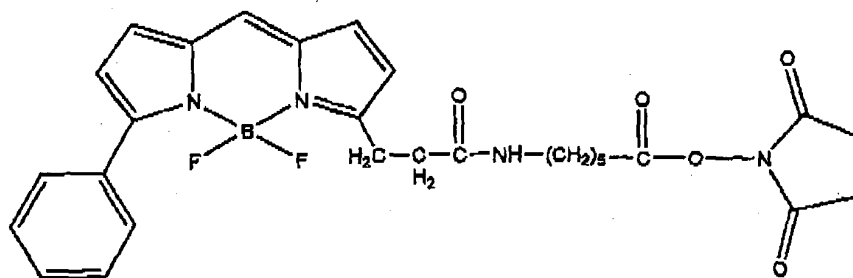
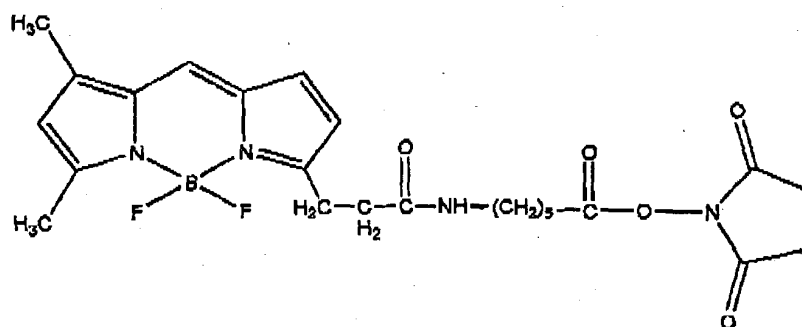
74. (New) A method, as claimed in Claim 73, wherein said solid support is made of solvent-resistant materials that are selected from the group consisting of nylon, poly(vinylidene difluoride), glass, plastics, and their derivatives.

75. (New) A method, as claimed in Claim 74, wherein said solid support is poly(vinylidene difluoride).

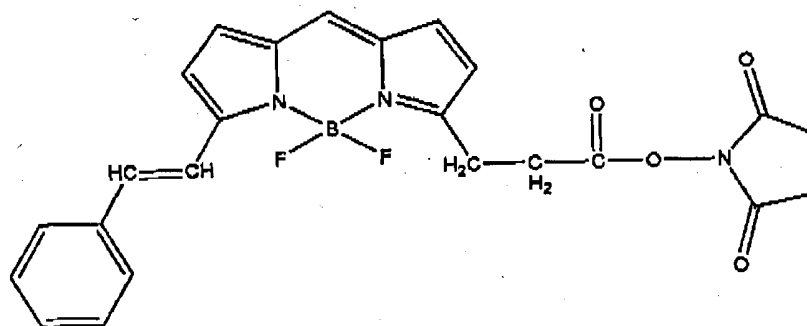
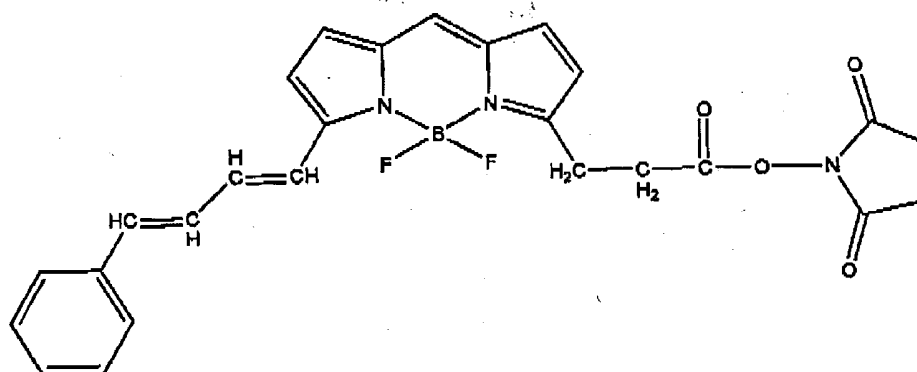
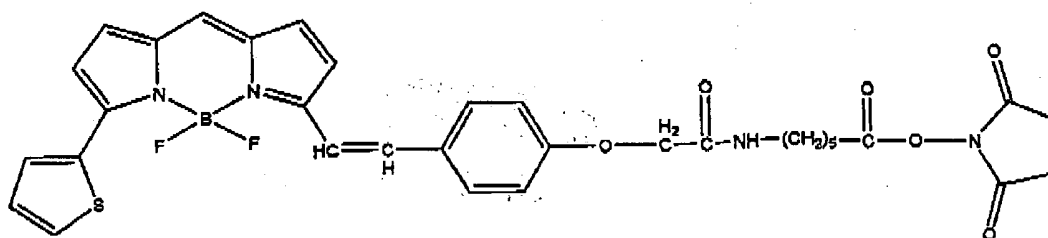
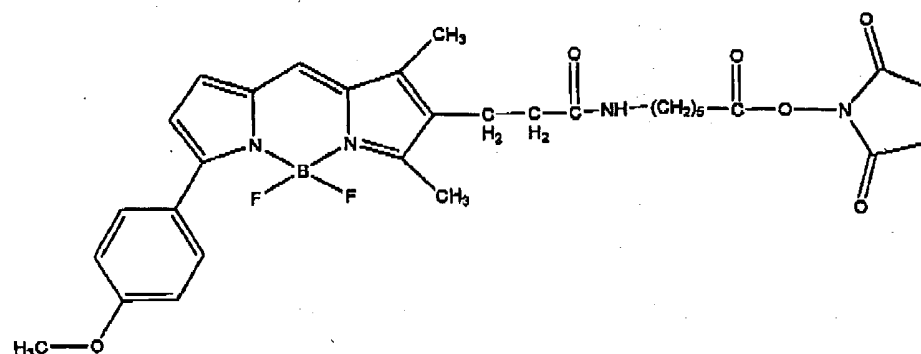
76. (New) A method, as claimed in Claim 75, wherein said dipyrrometheneboron difluoride dye is present in the labeling mixture at a concentration of about 5 micromolar to about 20 micromolar.

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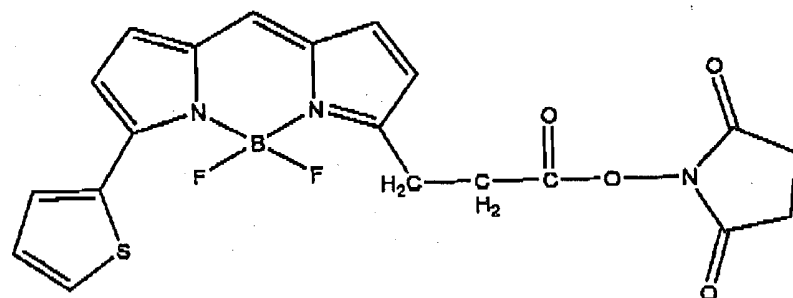
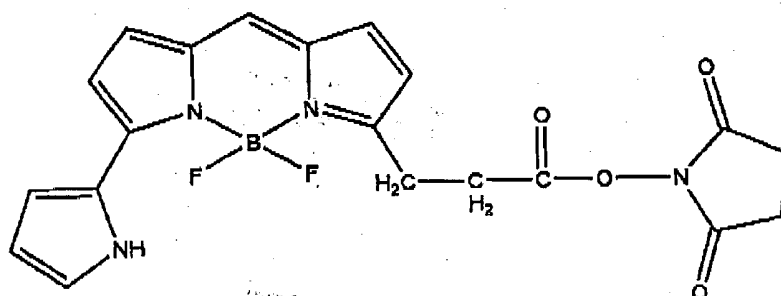
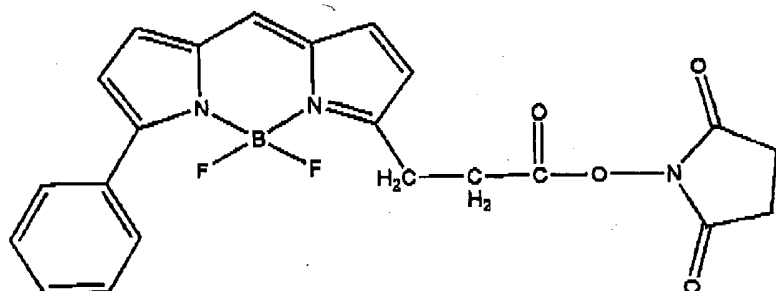
77. (New) A method, as claimed in Claim 76, further comprising adding a specific binding pair member that selectively binds to a target within said immobilized poly(amino acids) that is its complementary binding pair.
78. (New) A method, as claimed in Claim 77, where said specific binding pair member contains a label that is an enzyme, a fluorophore or a hapten.
79. (New) A method, as claimed in Claim 77, further comprising:  
adding a secondary complementary binding pair member that contains a label and that selectively binds to the specific binding pair member.
80. (New) A method, as claimed in Claim 76, wherein said dye is selected from the group consisting of



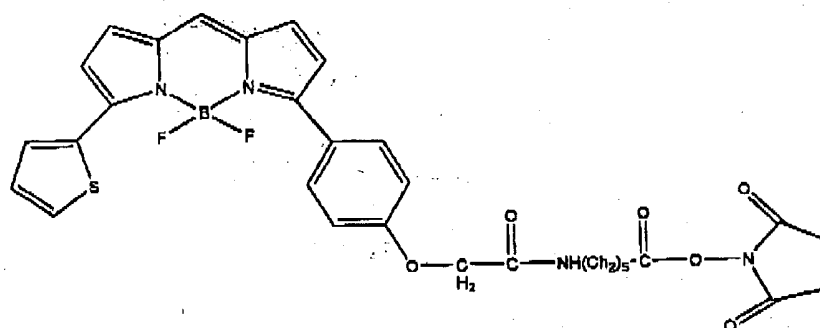
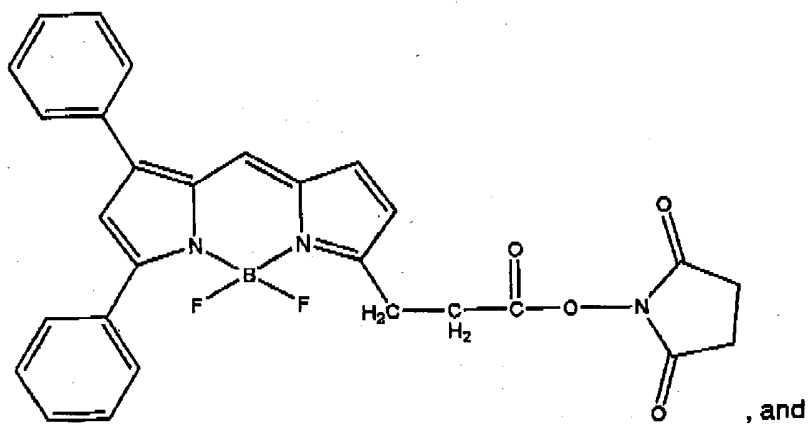
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### CONCLUSION

In view of the above amendments and remarks, it is submitted that this application is now ready for allowance. Early notice to this effect is solicited. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned at (541) 335-0203.

Respectfully submitted,

Date: July 2, 2004

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